IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Application of H. Van Urk, D.J. Mead, P.H. Morton, A.J. Cartwright, J. Cameron, D.J. Balance, M.G.J. Grandgeorge, S. Berezenko, J.R. Woodrow, D. Sleep, and J-L.B. Veron

Examiner: T.E. Strzelecka
Art Unit: 1637
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Process

(Atty. Docket No. P27,692 USA)

DECLARATION OF STEPHEN BEREZENKO UNDER 37 C.F.R. § 1.132

I, Stephen Berezenko, hereby declare the following:

1. I am an inventor named on the above-identified application (hereafter "the present application").

2. I am presently Research Director at Delta Biotechnology Limited, the assignee of the present application.

3. I have read the above-identified application and Applicant's Reply Under 37 C.F.R. § 1.112 To The Office Action Dated March 10, 2005 including the experimental data provided within the Reply.

4. The experiments that provided the data were conducted under my supervision. I am familiar with the procedures used and the results obtained by the experiments.

5. The claimed invention came about as a result of our further development of the albumin purification processes. These developments relate to end-stage "polishing" processes that can be applied to albumin that has already been partially purified in order to obtain even more highly purified albumin.

6. We found that partially purified albumin could be further purified by exposure to cation and anion exchange steps, wherein the cation exchange step is run in the negative mode with respect to albumin. In particular, we found that the negative mode cation exchange step operated more efficiently (*i.e.* allowed the recovery of more albumin and yet removed a greater proportion of impurities) when the albumin solution that was applied to the negative mode cation exchange step was highly concentrated. Moreover, we found that cation exchange chromatography that is run in the negative mode with respect to albumin could be used to separate non-glycosylated albumin from glycosylated albumin. Experiments were conducted to confirm these results.

7. In the first experiment, different concentrations of an impure albumin solution were applied to a cation exchange chromatography column operated in the negative mode with respect to albumin. The cation exchange column used was an SP-FF matrix packed into a 1.6 cm diameter column (Pharmacia XK16) to a bed height of 11 cm. The column was loaded and washed at a flow rate of 0.1 column volumes.min^{-1}; all other steps were performed at a flow rate of 0.5 column volumes.min^{-1}. The albumin-containing cation exchange flow through was collected and the yield and contaminant levels were measured. The yield was determined by GP-HPLC. The detected contaminated level was measured by a Con A assay. Concanavalin A (Con A) binds molecules, which contain α-D-mannopyranosyl, α-D-glucopyranosyl and sterically

related residues. In the Con A assay, 100mg samples of rHA are diluted in a pH5.5 buffer containing 200 mM sodium acetate, 85 mM sodium chloride, 2 mM magnesium chloride, 2 mM manganese chloride and 2 mM calcium chloride and passed through a 2mL Con A Sepharose affinity chromatography column. The amount of Con a binding protein recovered is quantified and calculated as % (w/w) of the protein loaded. The results of this experiment are shown in the following table.

Load Concentration	**Albumin yield %**	**Detected Contaminant Level %**
5 $g.L^{-1}$	39	0.17
10 $g.L^{-1}$	53	0.15
25 $g.L^{-1}$	67	0.10

8. The results of the first experiment indicate that at higher load concentrations, negative mode cation exchange chromatography provides for more efficient recovery of albumin (albumin recovery increases from 39% to 67%). Moreover, at the same time, higher load concentrations result in impurities being more efficiently removed (contaminant levels reduced from 0.17% to 0.10%).

9. In the second experiment, a 50 $g.L^{-1}$ albumin solution was applied to a cation exchange ("CE") chromatography column run in the negative mode with respect to albumin. The cation exchange column used was an SP-FF matrix packed into a 1.6 cm diameter column (Pharmacia XK16) to a bed height of 22 cm. The column was loaded and washed at a flow rate of 0.1 column volumes.min^{-1}; all other steps were performed at a flow rate of 0.5 column volumes.min^{-1}. The albumin-containing cation exchange flow through was collected. The glycosylated albumin content was measured both before and after being applied to the cation exchange column. The percentage of glycosylated

albumin was calculated by a Con A assay. The results of the experiment are shown in the following table.

Glycosylated albumin content	
Before CE treatment	After CE treatment
0.2%	0.07%

10. The results of this experiment indicate that cation exchange chromatography that is run in the negative mode with respect to albumin can be used to separate non-glycosylated albumin from glycosylated albumin. Glycosylated albumin is an undesirable contaminant.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 10 August 2005

Stephen Berezenko

Stephen Berezenko